Plant Physiol. (1980) 66, 8–12
0032-0889/80/66/0008/04$00.50/0

Metabolism of Specifically Labeled Glucose, Glucose 1-Phosphate, and Glucose 6-Phosphate via the Oxidative Pentose Phosphate Cycle in a Reconstituted Spinach Chloroplast System in Darkness and in the Light[1]

Received for publication September 4, 1979 and in revised form February 6, 1980

KLAUS J. LENDZIAN
Institut für Botanik und Mikrobiologie, Lehrstuhl für Botanik, Technische Universität, D-8000 München 2, Federal Republic of Germany

ABSTRACT

Using isolated spinach (hybrid 424) chloroplasts deprived of their envelopes (reconstituted chloroplast system), the metabolism of glucose, glucose 1-phosphate, and glucose 6-phosphate via the oxidative pentose phosphate cycle was analyzed. The activity of oxidative pentose phosphate cycle was monitored by continuous sampling of the CO_2 released during the decarboxylation process of 6-phosphogluconate.

The rate of CO_2 released in the dark from [C-1-^{14}C]glucose 6-phosphate was 4 to 6 micromoles per milligram chlorophyll per hour. A CO_2 release from the C-6 position of [C-6-^{14}C]glucose 6-phosphate was hardly measurable within 60 minutes of incubation. Glucose 1-phosphate was readily converted to glucose 6-phosphate without externally added glucose bisphosphate and was metabolized via the oxidative pentose phosphate cycle. The phosphorylation of glucose to glucose 6-phosphate was mediated by hexokinase present in the reconstituted system. This step was rate-limiting for the over-all reaction of the oxidative pentose phosphate cycle with glucose being the substrate (0.5 micromoles per milligram chlorophyll per hour). Addition of hexokinase increased the rate of CO_2 release to 5 micromoles per milligram chlorophyll per hour.

The flow of carbon through the oxidative pentose phosphate cycle was greatly reduced upon the addition of NADPH and ATP. Whereas NADPH inhibited the metabolism of [C-1-^{14}C]glucose 6-phosphate via the oxidative pentose phosphate cycle, ATP stimulated carbon flow into the 3-phosphoglycerate, dihydroxyacetone phosphate, and bisphosphates pools via the glycolytic pathway mediated by phosphofructokinase. This regulatory phenomenon could also be demonstrated with the reconstituted system undergoing a dark-light-dark transition. Data are presented indicating the conditions under which glucose 6-phosphate or glucose 1-phosphate are metabolized via the oxidative pentose phosphate cycle and the glycolytic pathway.

Two metabolic pathways exist for the oxidation of G-6-P[2] within the chloroplasts of higher plants (*e.g.* spinach, pea), the oxidative PPC (5, 14, 15) and the glycolytic pathway (12, 13). It has been proposed that the oxidative PPC yields pentose-monoP and NADPH, the latter needed for reductive biosynthetic pathways (7, 21). A complete oxidative PPC also yields triose-P and PGA. Both compounds are known to be the predominant species which pass through the chloroplast envelope (6). The oxidative PPC could thus become an important alternative to the glycolytic pathway by which G-6-P can be metabolized. The relative amounts of G-6-P metabolized via the oxidative PPC and the glycolytic pathway are not well established, nor are the conditions under which one or the other pathway is predominant. Recently, Kaiser and Bassham (9) have pointed out that the conversion of G-6-P to triose-P is ATP-dependent and thus occurs mainly via the glycolytic pathway. Their data were based on the use of [U-^{14}C]G-6-P and on the distribution of label among various intermediates of the chloroplast metabolism.

The most valid method of determining the contribution of the oxidative PPC to glucose metabolism has been developed by Katz *et al.* (10, 11), using glucose specifically labeled in the C-1 and the C-6 position. The present study describes the use of specifically labeled G-6-P, G-1-P, and glucose to estimate the rates of G-6-P metabolism via the oxidative PPC and glycolytic pathways. In addition, evidence is presented concerning the effectors determining the flow of carbon through the two pathways.

MATERIALS AND METHODS

Plant Materials. Spinach (*Spinacia oleracea* L., hybrid 424) (Ferry-Morse Seed Co., Mt. View, Calif.) was grown in a growth chamber at 30,000 lux with a 10-h light period at 21 C and a 14-h dark period at 8 C. Seedlings were planted in pumice stone gravel (2- to 10-mm particle diameter) which had been flooded with a superphosphate solution (1% w/v) and a trace element solution (3) before planting. The gravel was flooded every 3rd day with a Weihenstephan nutrient medium (24) for 10 min. Leaves could be harvested after 6–10 weeks of cultivation. This type of cultivation resulted in highly reproducible physiological reactions of chloroplasts.

Chloroplasts were isolated according to Jensen and Bassham (8) with the exception that solution B was employed as the grinding and resuspension medium (pH 7.2).

Reconstituted Chloroplast System. Freshly isolated chloroplasts (2 mg Chl) were spun down (0.5 min, 2,000*g*) and resuspended in 1–2 ml of solution C (pH 7.2) (8). The suspension was sonicated for 5 s at 0 C (Branson sonifier, 45 w) and centrifuged for 15 min at 20,000*g* (2 C). The resulting supernatant served as the stroma solution. The pellet (thylakoids) was washed once with and resuspended in solution C (pH 7.2). Lamellae and stroma solutions

[1] This work was supported by the Deutsche Forschungsgemeinschaft.

[2] Abbreviations: G-6-P: glucose 6-phosphate; PPC: pentose phosphate cycle; PGA: 3-phosphoglycerate; G-1-P: glucose 1-phosphate; HK: hexokinase; 6-PGluA: 6-phosphogluconate; DHAP: dihydroxyacetone phosphate; FBP: fructose bisphosphate; F-6-P: fructose 6-phosphate; SBP: sedoheptulose bisphosphate.

were adjusted to the same volume. The isolated chloroplast components were recombined to give a reconstituted chloroplast system (2). Details are given under "Results."

Experimental Conditions. The reconstituted system (0.5 ml final volume) containing 0.4 ml stroma solution (pH 7.2), spinach ferredoxin (0.1 mg), and lamellae (0.1 mg Chl) was placed in 15-ml round bottom flasks (Fig. 1). Each flask was darkened by shrouding it with a black lighttight bag which could be removed to study the light reactions of the lamellae. The flasks were sealed by serum stoppers, connected to a N_2 reservoir via a manifold and flushed with N_2 (5 min). After cessation of N_2 flushing, 0.5 ml KOH (10% w/v) was placed into the side arm via a hypodermic needle and the reaction initiated. Reactions having G-1-P or G-6-P as substrate were started by addition of 1 mM $NADP^+$; the substrates were added to the flasks prior to the N_2 flushing period. When glucose was the substrate all compounds (glucose, HK, $NADP^+$) were added to the system prior to the N_2 flushing period; the reaction was started by adding ATP.

All experiments were run at a temperature of 20 C and a shaking rate of 80 rpm.

Analysis. At various intervals, 0.05-ml aliquots were withdrawn from the KOH solution in the side arm and the radioactivity determined by scintillation counting. "Cold" KOH (0.05 ml) was added to the KOH solution immediately after each withdrawal to maintain a constant volume.

To monitor the flow of ^{14}C from G-6-P, G-1-P, and glucose into various intermediate compounds of the reductive and oxidative PPC, samples (0.1 ml) taken from the reaction mixture were diluted with 0.2 ml methanol, and analyzed by two-dimensional TLC (4) or descending paper chromatography (23) followed by radioautography and liquid scintillation counting. ATP was determined according to (29). Amounts of $NADP^+$ were determined in acidified (to destroy any NADPH present) and neutralized samples with the G-6-P/G-6-PDH system by measuring NADPH production. Chl determination was according to Vernon (30).

Chemicals. [C-1-^{14}C]Glucose, [C-6-^{14}C]glucose, [U-^{14}C]glucose, and [U-^{14}C]G-1-P were obtained from Amersham-Buchler, Braunschweig. [C-1-^{14}C]G-6-P, [C-6-^{14}C]G-6-P, and [U-^{14}C]G-6-P were generated from the nonphosphorylated compounds with HK (33 units; Sigma) and stoichiometric amounts of ATP. The G-6-P generated was separated from the reaction mixture by one-dimensional paper chromatography (20).

RESULTS

Results. The flow of carbon through the oxidative PPC was monitored via the rate of CO_2 release from the reaction mixture. Diffusion of CO_2 from the reaction compartment (stroma solution) into the KOH solution (Fig. 1) was affected by the speed at which the flasks were shaken. The diffusion rate increased with increasing shaking frequency up to 60 rpm. Above this frequency the shaking rate had no further effect on the diffusion rate.

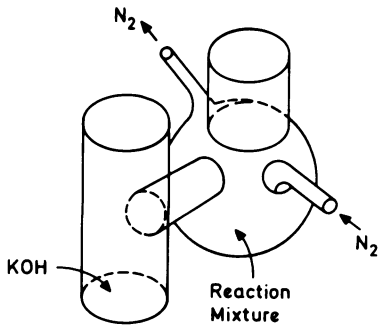

FIG. 1. The reaction flask used throughout the experiments. The reaction compartment and the KOH (side arm) compartment were sealed off by serum stoppers. The round bottom flask had a volume of 15 ml. The total volume of each reaction flask was 25 ml.

A logarithmic plot of the CO_2/HCO_3^- concentration remaining in the reaction mixture *versus* time indicated that the transfer of CO_2 exhibited first-order kinetics. However, at maximum diffusion rates first-order kinetics were not observed when the CO_2 concentration in the reaction mixture reached very low levels, *e.g.* after 40 min. Initial lag phases in the transition process were not observed, since the 0.6 mM $NaHCO_3$ and thus, the CO_2 concentration in the reaction mixture was high. However, such lag phases (lasting about 5–10 min) were measured during the experiments described in Figures 2–6. These lag phases were due to slow equilibration of CO_2 between the CO_2-producing reaction mixture and the gaseous phase. These lag phases had no impact on the following linear rates of CO_2 release.

Chloroplasts deprived of their envelopes metabolized G-6-P via the oxidative PPC, yielding CO_2 (Fig. 2). The system released CO_2 from [C-1-^{14}C]G-6-P at a steady-state rate of 5.3 μmol/mg Chl·h. CO_2 release from [C-6-^{14}C]G-6-P was hardly detectable. The 6-PGluA pool was subsequently labeled after the start of the experiment and reached a steady-state level within 30–40 min. During steady-state the concentration of 6-PGluA was about 0.2 mM. The omission of $NADP^+$ almost completely prevented the CO_2 release from [C-1-^{14}C]G-6-P and prevented labeling of the 6-PGluA pool. This was also true when G-6-P was replaced by glucose, HK, and ATP (Fig. 3). In the presence of $NADP^+$, CO_2 was released at a rate (4.8 μmol/mg Chl·h almost identical with the rate obtained with G-6-P. The omission of HK from the reconstituted system resulted in rates of CO_2 release which were around 0.5 μmol/mg Chl·h but which were significantly higher than those measured in the absence of $NADP^+$. ATP was essential for the over-all reaction as was $NADP^+$.

The reconstituted system was capable of metabolizing G-1-P via the oxidative PPC (Fig. 4). The highest rates were obtained when G-1-P and $NADP^+$ were the only additives to the system in

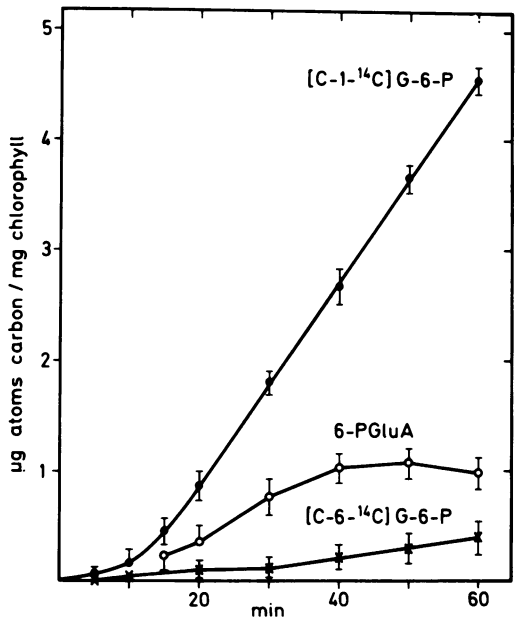

FIG. 2. Metabolism of G-6-P specifically labeled at the C-1 or C-6 position via the oxidative PPC in a reconstituted spinach chloroplast system during a dark period. (●——●, ×——×): Release of CO_2 from the specifically labeled G-6-P indicated; (○——○): pool size of 6-PGluA determined simultaneously with the CO_2 release experiments. The reaction mixture (pH 7.2) contained G-6-P (2 mM, specific radioactivity 3.6 μCi/μmol) and $NADP^+$ (1 mM). Values are averages of four determinations. SDs are given as vertical bars.

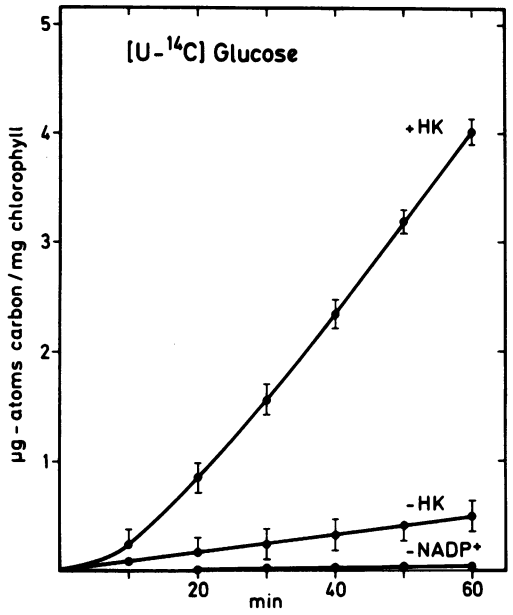

FIG. 3. The release of CO_2 from [U-^{14}C]glucose metabolized via the oxidative PPC in a reconstituted spinach chloroplast system during a dark period and in the presence and absence of HK (33 units) and $NADP^+$ (1 mM). Additionally, the reaction mixture (pH 7.2) contained glucose (2 mM, specific radioactivity 18.7 µCi/µmol) and ATP (2 mM). Values are averages of five determinations. SDs are given as vertical bars.

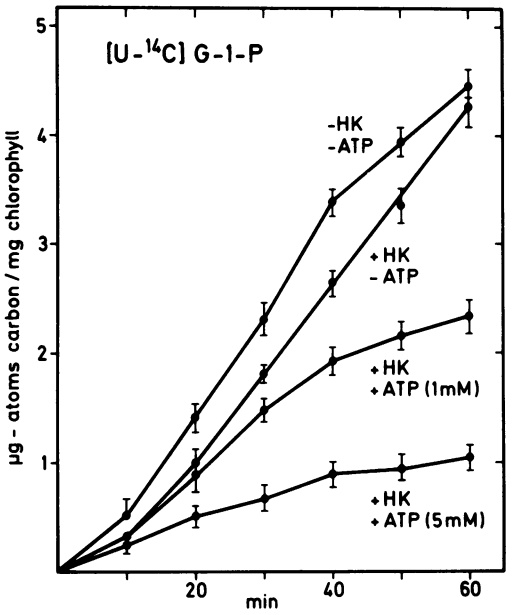

FIG. 4. The release of CO_2 from [U-^{14}C]G-1-P metabolized via the oxidative PPC in a reconstituted spinach chloroplast system during a dark period and in the presence and absence of HK (33 units) and ATP (0, 1, 5 mM). Additionally, the reaction mixture (pH 7.2) contained G-1-P (2 mM, specific radioactivity 3.0 µCi/µmol) and $NADP^+$ (1 mM). Values are averages of four determinations. SDs are indicated by vertical bars.

the dark (3–6 µmol/mg Chl·h). The addition of glucose-1,6-bisP had no effect on the rate. Added HK affected the initial CO_2 release to some extent. A pronounced inhibition of G-1-P metabolism via the oxidative PPC was observed when ATP had been added, especially at high concentrations (5 mM). The degree of inhibition increased during prolonged incubation periods up to 60 min. The CO_2 release, however, was never suppressed completely. On the contrary, when the ATP pool diminished to a certain level G-6-P metabolism via the oxidative PPC became enhanced once more (Fig. 5).

Taking the CO_2 release from the C-1 position of G-6-P as the indicator for the operation of the oxidative PPC and the changes of the pool sizes of PGA, DHAP, and the bisphosphates (SBP and FBP) as representing the activity of the glycolytic pathway, it can be shown that the distribution of G-6-P between these two pathways was determined by the presence or absence of NADPH (*e.g.* the NADPH/$NADP^+$ ratio) and ATP (Table I). At optimum assay conditions for the oxidative PPC (G-6-P = 2 mM; $NADP^+$ = 1 mM; no ATP) carbon flow via the oxidative PPC was about 3 times that via the glycolytic pathway. The glycolytic activity did not change when $NADP^+$ was omitted. Both NADPH and ATP added either separately or simultaneously to the test system shifted the CO_2 release to glycolytic activity ratios. Whereas NADPH decreased the CO_2 release without affecting glycolysis, ATP af-

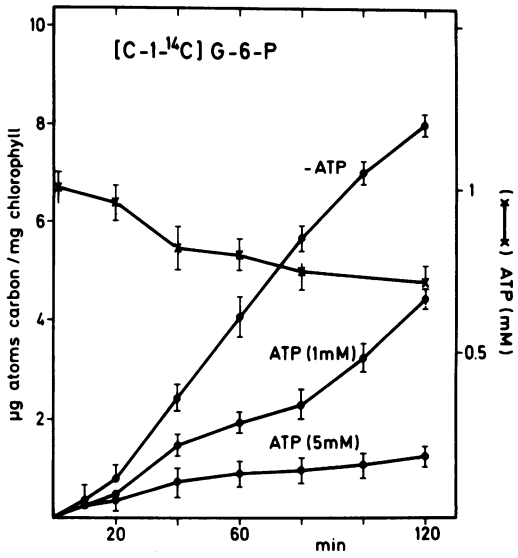

FIG. 5. The release of CO_2 from [C-1-^{14}C]G-6-P metabolized via the oxidative PPC in a reconstituted spinach chloroplast system during a dark period in the presence and absence of ATP (0, 1, 5 mM). Additionally, the reaction mixture (pH 7.2) contained G-6-P (2 mM, specific radioactivity 3.6 µCi/µmol) and $NADP^+$ (1 mM). The ATP pool (1 mM ATP added at the beginning of the experiment) was monitored throughout the experiment (×——×). Values are averages of five determinations. SDs are given by vertical bars.

Table I. *Distribution of [C-1-^{14}C]G-6-P between the Oxidative PPC (Determined as CO_2 Release) and the Glycolytic Pathway (Determined as Pool Sizes of PGA + DHAP + BisP) in a Reconstituted Spinach Chloroplast System after a 30-Min Dark Period*

Treatment	CO_2 Release	PGA + DHAP + BisP	Ratio	% Metabolized by the Oxidative PPC
	µmol/mg Chl			
Control[a]	1.95	0.61	3.20	76
−$NADP^+$	0.05	0.67	0.07	7
+1 mM NADPH	0.91	0.63	1.44	59
+2 mM NADPH	0.03	0.72	0.04	4
+1 mMATP	1.07	1.08	0.99	50
+5 mM ATP	0.35	1.89	0.19	16
+1 mM NADPH + 5mM ATP	0.07	2.19	0.03	3

[a] The control contained G-6-P (2 mM, specific radioactivity 3.6 µCi/µmol) and $NADP^+$ (1 mM). The pH value was adjusted to pH 7.2.

fected both pathways in that carbon flow through the glycolytic pathway was enhanced at the expense of the oxidative PPC.

This regulatory phenomenon could also be partly demonstrated when the reconstituted system had been exposed to dark-light-dark transitions (Fig. 6). In the light the system reduced $NADP^+$ resulting in $NADPH/NADP^+$ ratios around 1. As soon as the light was turned on the rate of CO_2 release from [C-1-^{14}C]G-6-P decreased and approached almost zero after 20 min of illumination. A switch from light to the dark immediately resulted in a regaining of the previously observed dark activity of the oxidative PPC. The DHAP pool reached almost steady-state size after a 20-min dark period but increased upon illumination. The transition from light to darkness induced almost no change of the steady-state pool size.

In the last experiment, ADP, normally an additive to the complete reconstituted system, was omitted to avoid refixation of the CO_2 via the reductive PPC. Ferredoxin was essential to bring about the effect described in Figure 6. In a further experiment similar to that in Figure 6, G-6-P was replaced by [U-^{14}C]G-1-P. The results were the same as in Figure 6 and are not shown.

DISCUSSION

To evaluate the contribution of the oxidative PPC to the metabolism of chloroplasts in the dark it is necessary to have quantitative information on the flow of carbon via this and alternative pathways. The use of specifically labeled glucose or its phosphorylated derivatives G-1-P and G-6-P to study the characteristics of metabolic patterns for hexose utilization by chloroplasts requires a number of assumptions (10).

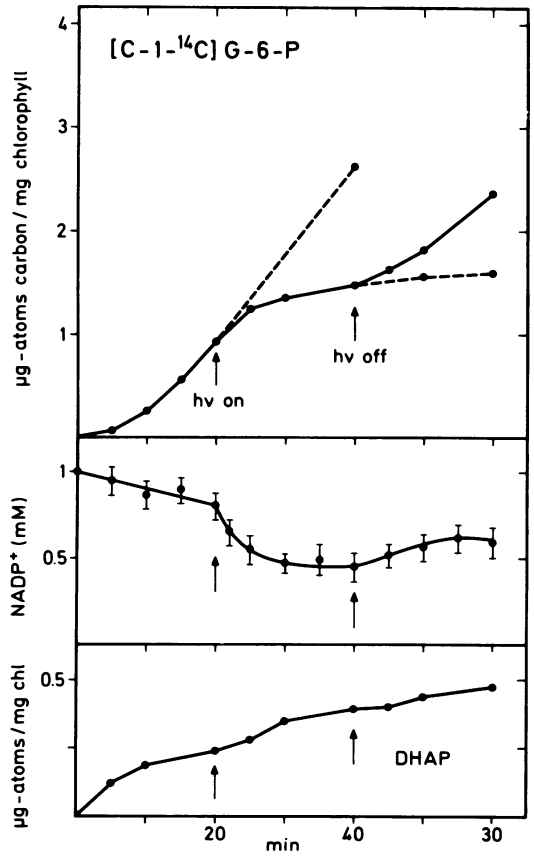

FIG. 6. The release of CO_2 from [C-1-^{14}C]G-6-P and the change of the pool sizes of $NADP^+$ and DHAP during a dark-light-dark transition in a reconstituted spinach chloroplast system. The reaction mixture (pH 7.2) contained G-6-P (2 mM, specific radioactivity 3.6 µCi/µmol) and $NADP^+$ (1 mM initial concentration). Transitions are indicated by arrows. Dotted lines represent the data of controls not subjected to the transition conditions. SDs where given were calculated from four determinations and are indicated by vertical bars.

1. That only the oxidative PPC and the glycolytic pathway are operative in the system employed (9). The distribution of the ^{14}C-label among metabolites separated by chromatography gave no indications that there are other pathways by which glucose-P could be utilized.

2. That CO_2 released by the decarboxylation of 6-PGluA is not recycled by the reconstituted system. Most of the experiments were run under dark conditions. In the case of the dark-light-dark transition experiment ADP and ATP were omitted. $^{14}CO_2$ was not fixed into acid-stable products in the light or in darkness when the G-6-P dehydrogenase mediated step was inhibited by high $NADPH/NADP^+$ ratios (data not shown; *cf.* 17) and/or by photoreduction (1).

3. That F-6-P is one product of the oxidative PPC. Its pool is easily equilibrated with the G-6-P pool, the equilibration being mediated by P-glucose isomerase. This equilibration could result in a dilution of the specific activity of the G-6-P pool leading to erroneous estimations of the activity of the oxidative PPC. The data given in Figure 2 point to the absence of such recycling as the rate of CO_2 release from [C-1-^{14}C]G-6-P remained constant over a period of 60 min.

4. That the fructose bisphosphatase was inhibited under dark conditions (32).

There is evidence that a direct decarboxylation of G-6-P is solely mediated by the oxidative PPC within the chloroplasts. Thus it is possible to calculate the contribution of the oxidative PPC to the catabolism of G-6-P by expressing the fraction:

$$\frac{CO_2 \text{ released from [C-1-}^{14}\text{C]G-6-P}}{\text{total G-6-P metabolized}}$$

The release of CO_2 from [C-1-^{14}C]G-6-P clearly demonstrates the operation of the oxidative PPC in chloroplasts and complements the evidence of earlier investigations (5, 9, 14, 15). Under optimal test conditions G-6-P is metabolized in the dark via the oxidative PPC with rates as high as 4–6 µmol/mg Chl·h. Similar rates were measured assaying G-6-P dehydrogenase directly (17, 18) suggesting that the activity of this key enzyme is rate-limiting. At the same time, only 25% of the total G-6-P metabolized by the reconstituted system was found in glycolytic intermediates (Table I, line 1). Under steady-state conditions the G-6-P pool is equilibrated with the F-6-P pool (Keq = 0.67; unpublished results), the equilibration being mediated by P-glucose isomerase. Thus the two enzymes G-6-P dehydrogenase and P-fructokinase play a crucial role in the metabolism of hexose monophosphates via the two pathways. Whereas G-6-P dehydrogenase activity is mainly determined by the $NADPH/NADP^+$ ratio within the stroma (17, 18, 31) and is not affected by ATP (16, 19), P-fructokinase appears to be an enzyme with highly complex regulatory characteristics (13). The irreversible step from F-6-P to FBP depends on ATP. Thus it can be stated, as supported by the data described, that the flow of carbon through the two pathways is mainly determined by the $NADPH/NADP^+$ ratio and the ATP available. Briefly, G-6-P metabolism depends on the $NADP^+$/ATP ratio in the stroma. Other effectors such as Pi (26), ADP, and PGA (13) also interact with P-fructokinase *in vivo*. The observation of Kaiser and Bassham (9) that increasing amounts of ATP shift G-6-P utilization via the glycolytic breakdown at the expense of G-6-P utilization via the oxidative PPC is supported by the data presented. A small decrease of the ATP pool (1 mM) can alter this situation to result in an increase of the oxidative PPC activity (Fig. 5). The $NADP^+$ available determines the activity of the oxidative PPC, whereas ATP (essential for P-fructokinase activity) determines the flow of carbon into the triose-P sink and thus the amount of G-6-P available for the oxidation of hexose-monoP (Table I). When,

under light conditions, the NADPH/$NADP^+$ ratio reaches values of approximately 1 (Fig. 6), the oxidative PPC is switched off at the G-6-P dehydrogenase-mediated step (17) and G-6-P can only be metabolized via the P-fructokinase-mediated step, depending on the activity of this enzyme.

Carbon can be fed into the G-6-P pool from G-1-P and glucose. The mutation of G-1-P to G-6-P was never rate-limiting under the experimental conditions employed, indicating that P-glucomutase and its co-substrate glucose bisP are constituents of reconstituted chloroplasts. HK was present in the chloroplasts with an activity of 0.5 μmol glucose esterified/mg Chl·h. In the over-all reaction glucose → CO_2 release, the esterification of glucose was the rate-limiting step and could be overcome by adding HK. The presence of HK in spinach chloroplasts has been reported by Peavey *et al.* (22), but not found by Stitt *et al.* (28) for pea chloroplasts.

Evidence is accumulating that both a phosphorolytic (22, 27) and an amylolytic (25) degradation of starch are possible in spinach chloroplasts. Products of both pathways can be converted to G-6-P by the stroma. Depending on the reduction charge and the ATP available G-6-P is metabolized via the oxidative PPC or the glycolytic pathway. However, it must be emphasized that the conversion of G-6-P to exportable triose-P via the oxidative PPC is less effective. Only two triose-P but six CO_2, four G-6-P, and 12 NADPH result from six G-6-P molecules per cycle.

Acknowledgments—The author is grateful to Prof. H. Ziegler and Dr. J. Schönherr for helpful discussions and critical reading of the manuscript and to Ms. E. König for skilled technical assistance.

LITERATURE CITED

1. Anderson LE, TCL Ng, KEY Park 1974 Inactivation of pea leaf chloroplastic and cytoplasmic glucose-6-phosphate dehydrogenase by light and dithiothreitol. Plant Physiol 53: 835–839
2. Bassham JA, G Levine, J Forger 1974 Photosynthesis *in vitro*. Plant Sci Lett 2: 15–21
3. Ellis C, MW Swaney 1953 Soilless Growth in Plants, Ed 2. Reinhold, New York
4. Feige B, H Gimmler, WD Jeschke, W Simonis 1969 Eine Methode zur dünnschichtchromatographischen Auftrennung von ^{14}C- und ^{32}P-markierten Stoffwechselprodukten. J Chromatogr 41: 80–90
5. Heber U, MA Hudson, UW Hallier 1967 Lokalisation von Enzymen des reduktiven und dem oxydativen Pentosephosphatzyklus in den Chloroplasten und Permeabilität der Chloroplasten-Membranen gegenüber Metaboliten. Z Naturfsch 22b: 1200–1215
6. Heldt HW 1976 Metabolite transport in intact spinach chloroplasts. *In* J Barber, ed. The Intact Chloroplast. Elsevier, Amsterdam, 215–234
7. Holzer H, I Witt 1968 Beschleunigung des oxydativen Pentosephosphatcyclus in Hefezellen durch Ammoniumsalze. Biochim Biophys Acta 38: 163–164
8. Jensen RG, JA Bassham 1966 Photosynthesis in isolated chloroplasts. Proc Nat Acad Sci USA 56: 1095–1101
9. Kaiser WM, JA Bassham 1979 Carbon metabolism of chloroplasts in the dark: oxidative pentose phosphate cycle versus glycolytic pathway. Planta 144: 193–200
10. Katz J, HG Wood 1963 The use of $^{14}CO_2$ yields from glucose-1- and -6-^{14}C for the evaluation of the pathway of glucose metabolism. J Biol Chem 238: 517–523
11. Katz J, BR Landau, GE Bartsch 1966 The pentose cycle, triose phosphate isomerization, and lipogenesis in rat adipose tissue. J Biol Chem 241: 727–740
12. Kelly GJ, E Latzko 1977 Chloroplast phosphofructokinase. I. Proof of phosphofructokinase activity in chloroplasts. Plant Physiol 60: 290–294
13. Kelly GJ, E Latzko 1977 Chloroplast phosphofructokinase. II. Partial purification, kinetic and regulatory properties. Plant Physiol 60: 295–299
14. Krause GH, JA Bassham 1969 Induction of respiratory metabolism in illuminated *Chlorella pyrenoidosa* and in isolated spinach chloroplasts by the addition of vitamin K_5. Biochim Biophys Acta 172: 553–565
15. Lendzian KJ, H Ziegler 1970 Über die Regulation der Glucose-6-phosphat-Dehydrogenase in Spinatchloroplasten durch Licht. Planta 94: 27–36
16. Lendzian KJ, H Ziegler 1972 Effect of light on glucose-6-phosphate dehydrogenase in spinach chloroplasts. *In* G Forti, M Avron, A Melandri, eds, Proc 2nd Int Congr Photosyn Res, Vol 3. Dr W Junk, The Hague, 1831–1838
17. Lendzian KJ, JA Bassham 1975 Regulation of glucose-6-phosphate dehydrogenase in spinach chloroplasts by ribulose-1,5-diphosphate and NADPH/$NADP^+$ ratios. Biochim Biophys Acta 396: 260–275
18. Lendzian KJ 1978 Interactions between magnesium ions, pH, glucose-6-phosphate, and NADPH/$NADP^+$ ratios in the modulation of chloroplast glucose-6-phosphate dehydrogenase *in vitro*. Planta 141: 105–110
19. Lendzian KJ, H Ziegler, N Sankhla 1978 Effect of phosphon-D on photosynthetic light reactions and on reactions of the oxidative and reductive pentose phosphate cycle in a reconstituted spinach (*Spinacia oleracea* L.) chloroplast. system. Planta 141: 199–204
20. Mortimer DC 1952 Paper chromatographic separation of some biologically important phosphate esters. Can J Chem 30: 653–660
21. Osmond CB, T Ap Rees 1969 Control of the pentose-phosphate pathway in yeast. Biochim Biophys Acta 184: 35–42
22. Peavey DG, M Steup, M Gibbs 1977 Characterization of starch breakdown in the intact spinach chloroplast. Plant Physiol 60: 305–308
23. Pedersen TA, MR Kirk, JA Bassham 1966 Light-dark transitions in levels of intermediate compounds during photosynthesis in air-adapted *Chlorella*. Physiol Plant 19: 219–231
24. Penningsfeld F, P Kurzmann 1966 Hydrokultur und Torfkultur. Ulmer, Stuttgart, 39
25. Pongratz P, E Beck 1980 Diurnal oscillation of amylolytic activity in spinach chloroplasts. Plant Physiol. In press
26. Steup M, DG Peavey, M Gibbs 1976 The regulation of starch metabolism by inorganic phosphate. Biochem Biophys Res Commun 72: 1554–1561
27. Steup M, E Latzko 1979 Intracellular localization of phosphorylases in spinach and pea leaves. Planta 145: 69–75
28. Stitt M, PV Bulpin, T Ap Rees 1978 Pathway of starch breakdown in photosynthetic tissues of *Pisum sativum*. Biochim Biophys Acta 544: 200–214
29. Strehler BL 1970 ATP und Creatinphosphat. *In* HU Bergmeyer, ed, Methoden der enzymatischen Analyse. Chemie, Weinheim, 2036–2050
30. Vernon LP 1960 Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. Anal Chem 32: 1144–1150
31. Wildner GF 1975 The regulation of glucose-6-phosphate dehydrogenase in chloroplasts. Z Naturforsch 30c: 756–760
32. Wolosiuk RA, BB Buchanan 1977 Thioredoxin and glutathione regulate photosynthesis in chloroplasts. Nature 266: 565–567